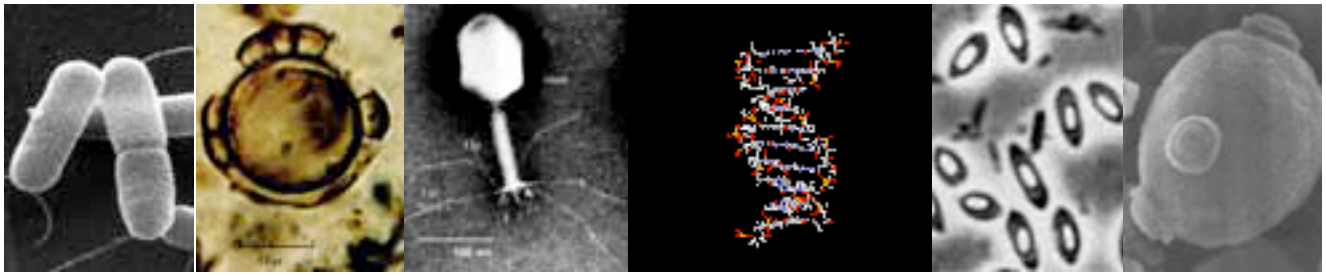


Microbial metabolism

General Microbiology - Lectures 5-6

Cañada College - Fall 2008

Instructor: Tamas Torok, Ph.D.

Topics for two days

- Laws of thermodynamics
- Metabolism in general
- Enzymes
- Energy production/catabolism
  - substrate-level phosphorylation
    - glycolysis and alternatives
    - fermentation
  - oxidative phosphorylation
  - photo-phosphorylation
- Anabolism

Basic energy concept

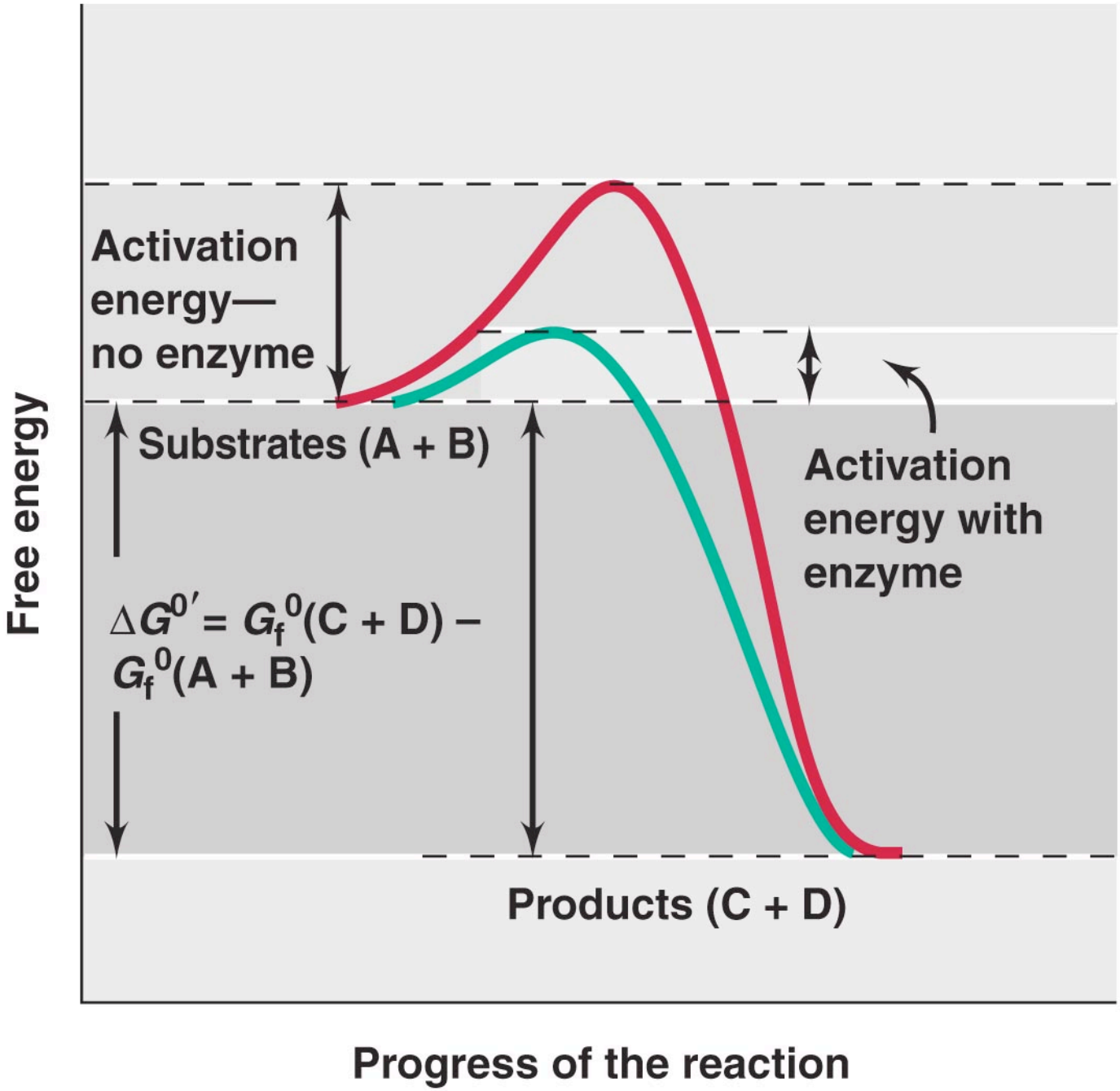
- **Cells**
  - **open, non-equilibrium systems**
- **First law of thermodynamics**
  - ***conservation of energy*** **- energy can neither be created nor destroyed in the universe**
- **Second law of thermodynamics**
  - **energy spontaneously disperses (if it is not hindered from doing so)**
  - ***predicts the probability*** **of the dispersal**
  - **entropy change measures *how much* or *how widely* energy is spread out in a process**

Example photosynthesis

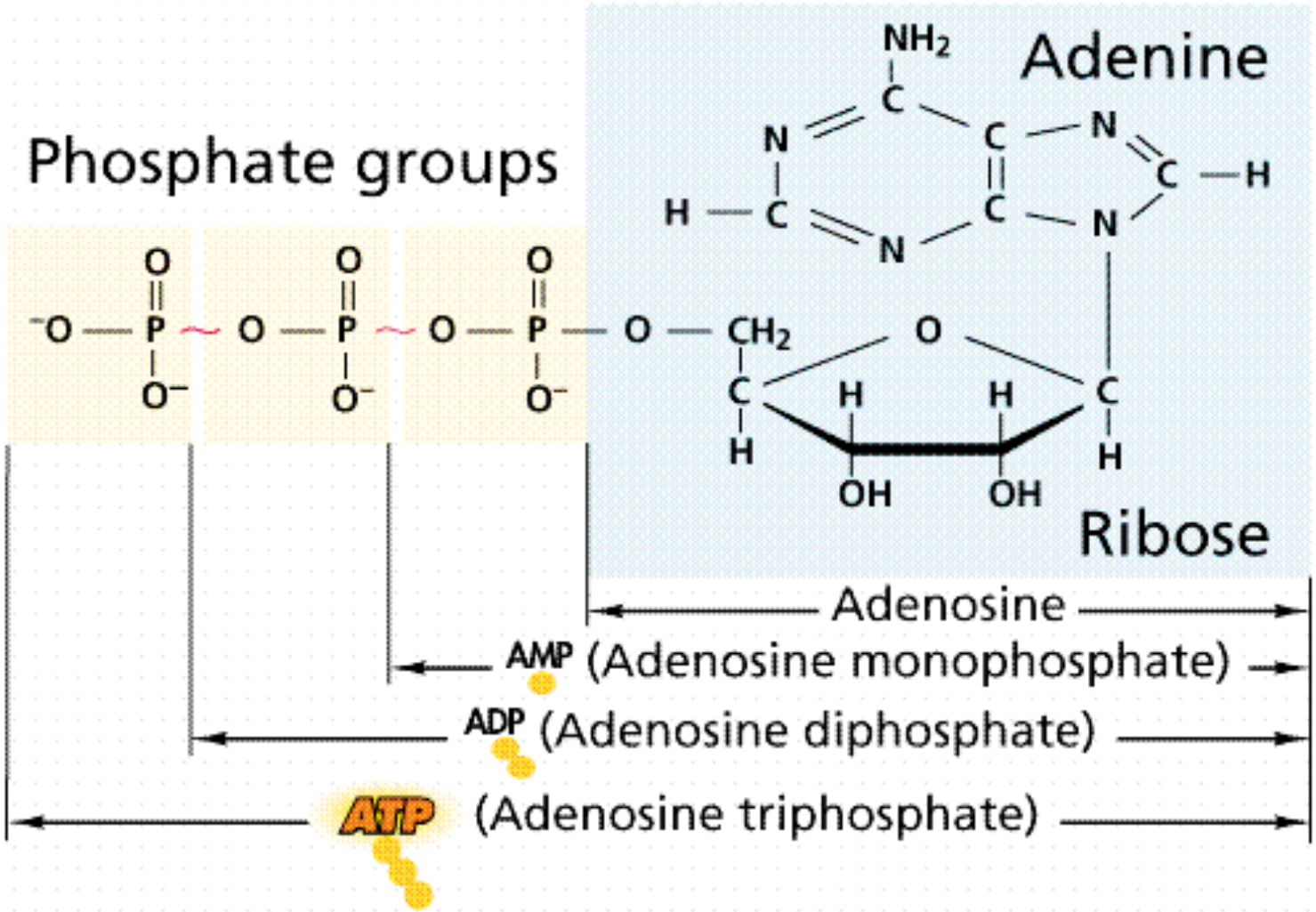
- **Energy dispersal and diversion of part of the energy flow**
  - **photosynthesizing organisms take certain wavelengths of the sun's dispersing energy, plus carbon dioxide and water, and make new chemical compounds that are more complex and more energy-containing**
  - **photosynthesis is in the 30% range**
    - **70% of the sun's energy is dispersed to the environment (net entropy increase)**

How can life exist?

- Activation energy is the innate obstacle to the second law of thermodynamics in chemical reactions
- Role of biological catalysts
- Metastable patterns among biological structures and processes are highly regulated by a vast variety of feedback systems
- Example
  - storage of energy in ATP is contrary to the predictions of the second law
    - energy within the bonds of the ATP molecule is kept from being dispersed by activation energy barriers until life needs it for a reaction


Activation energy— no enzyme
Substrates (A + B)
$\Delta G^{0'} = G_f^0(C + D) - G_f^0(A + B)$
Activation energy with enzyme
Products (C + D)
Free energy
Progress of the reaction

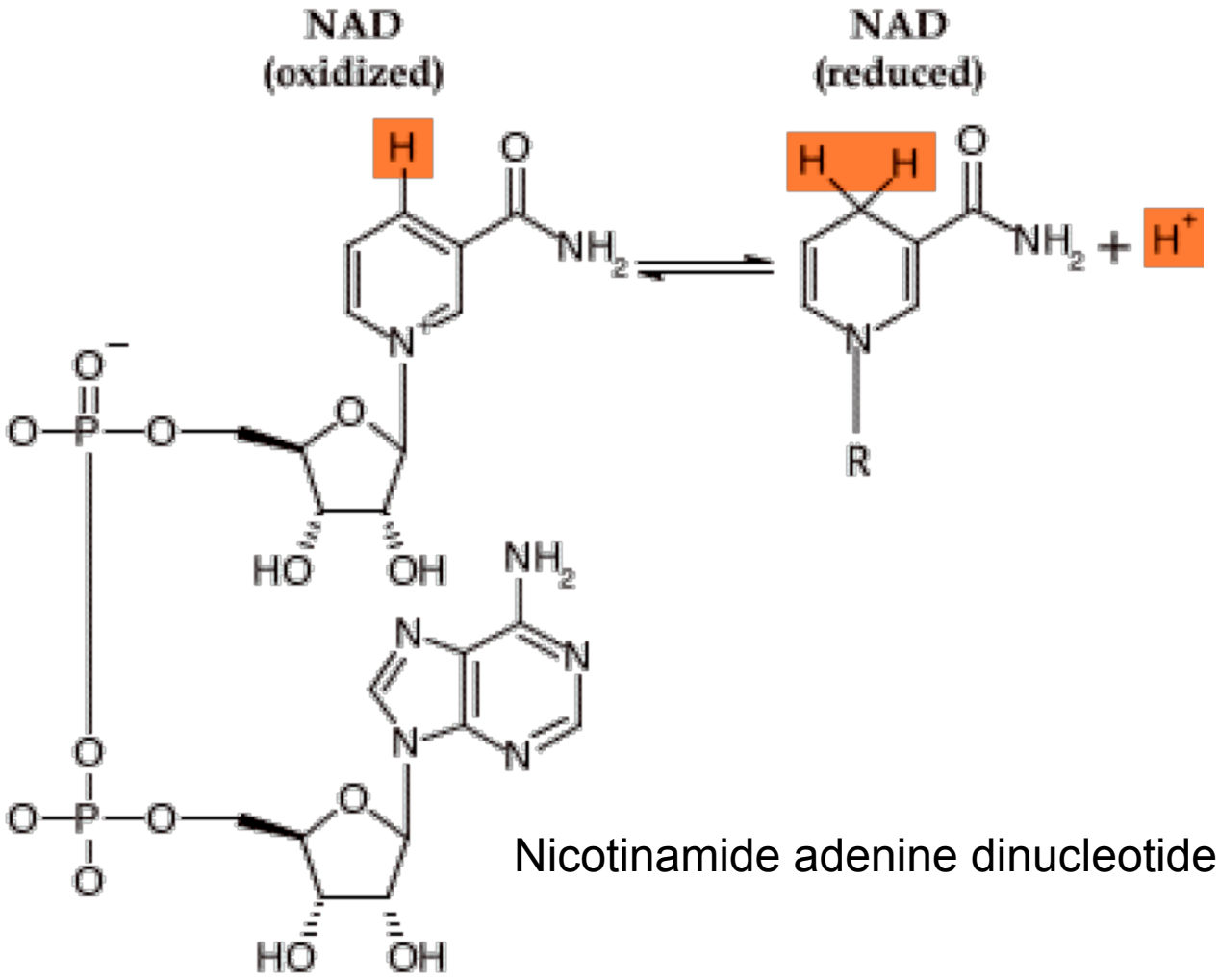
ATP (adenosine triphosphate)



Energy production

- **Redox reaction**
  - **oxidation: loss of electrons**
  - **reduction: gain of electrons**
- **Each molecule has the potential to donate and accept electrons from another molecule**

NAD/NADH



Nicotinamide adenine dinucleotide

Second law of thermodynamics

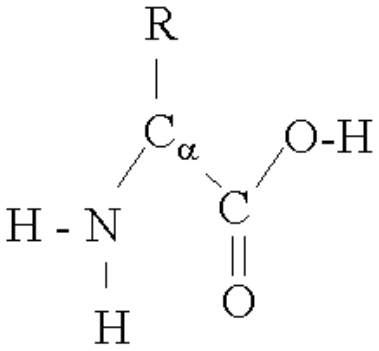
- **Greatest good - because of the second law, life is possible**
  - life can take in concentrated energy and use some of that energy to synthesize "uphill" complex biochemicals and to run highly regulated interdependent processes, including millions of non-spontaneous reactions
- **Biggest bad - because of the second law, life is always threatened**
  - non-spontaneous metabolic reactions are metastable
  - life cannot function unless a multitude of "molecular machines" and biochemical cycles operate synchronically in using energy to oppose second law predictions

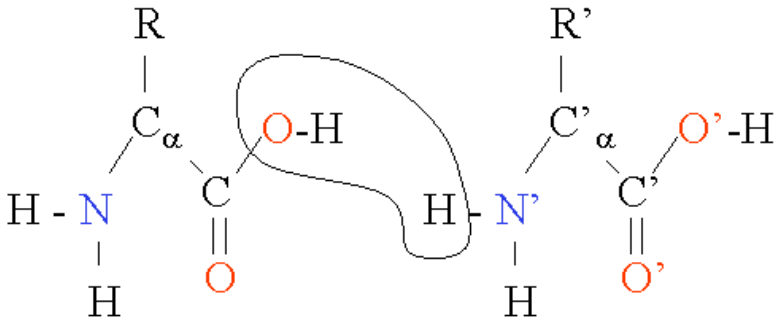
Metabolism

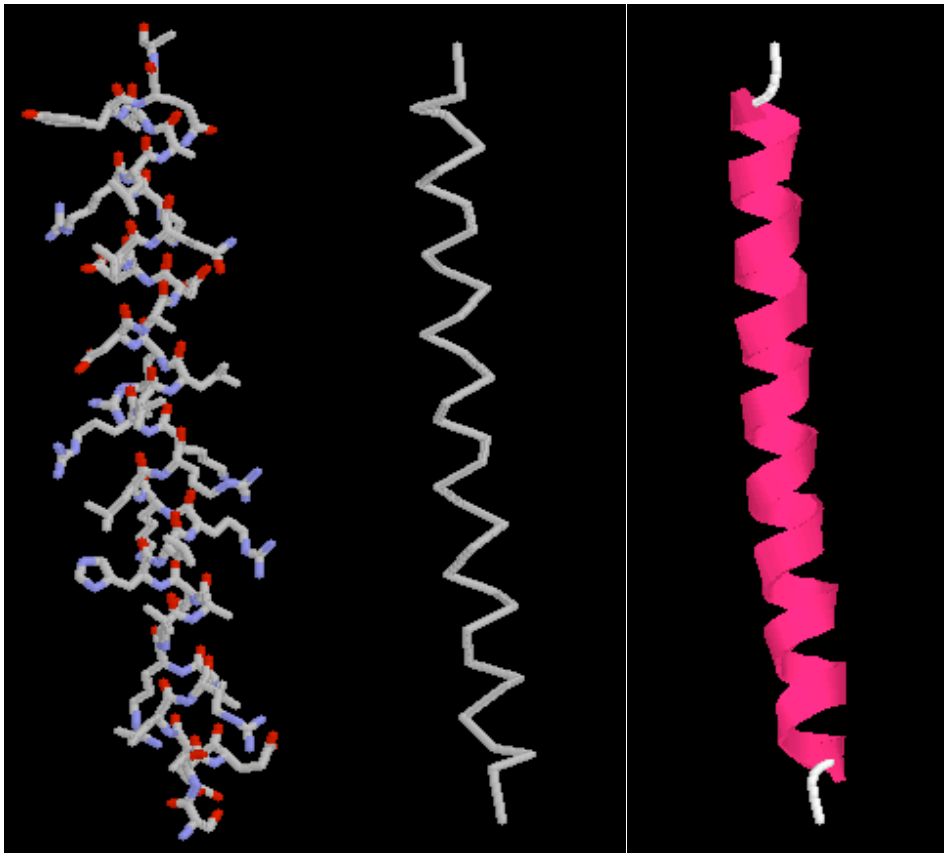
- **Metabolism**
  - sum total of all reactions that occur in a cell
- **Catabolic reactions**
  - break down of complex molecules into smaller, simpler molecules with the release of energy and reducing power (electrons)
- **Anabolic reactions**
  - synthesis of complex molecules from simpler ones
  - requires energy and reducing power (electrons) to form cell structures
- **Catabolic and anabolic reactions**
  - coupled, highly regulated, interdependent, and ***simultaneous***

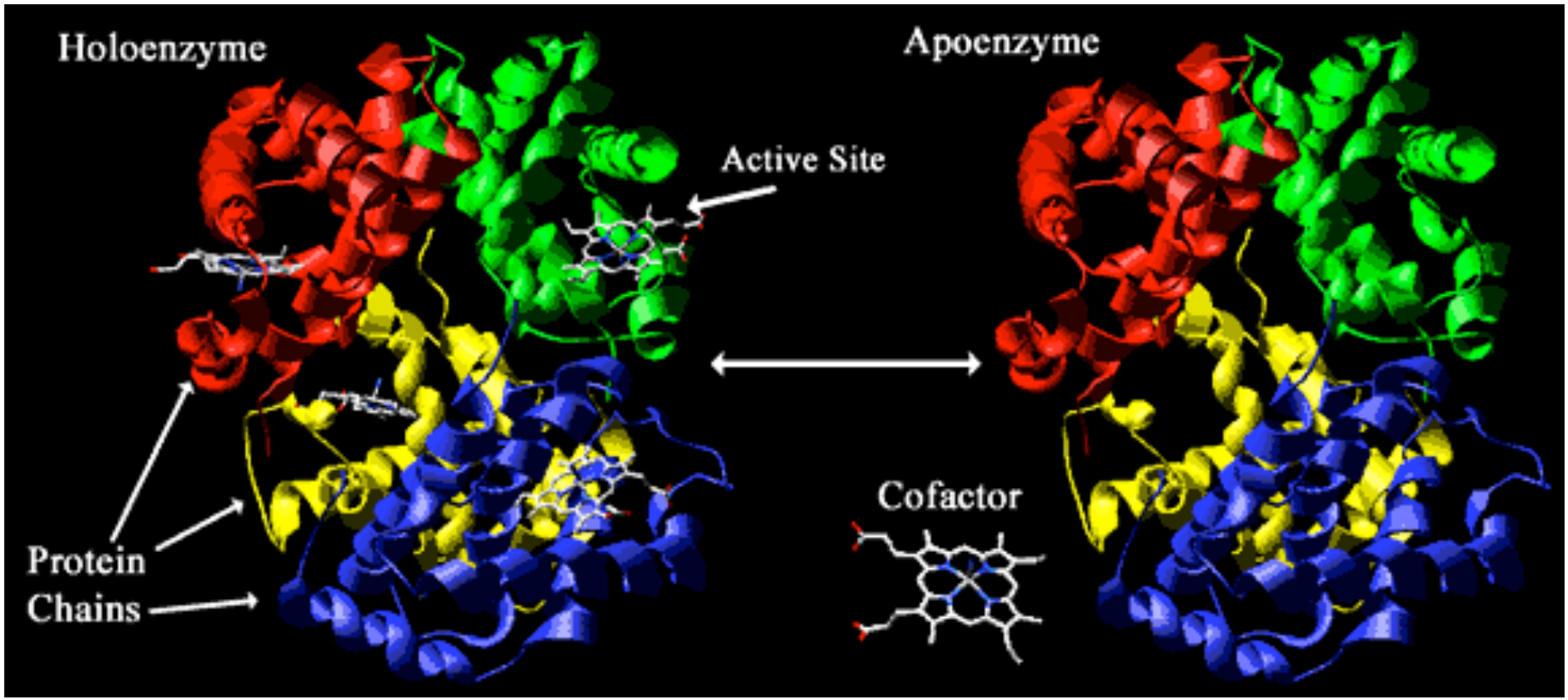
Enzymes

- What is an enzyme?
- Functional enzyme
- Mechanism of enzymatic reaction
  - "generic" version
  - one enzyme - many substrates
  - many enzymes - one substrate
  - classes of enzymes
- Regulation of enzymes
  - via synthesis (topic for a later evening)
  - via activity


R
Cα
O-H
H - N
C
O
H


R
R'
Cα
O-H
C'α
O'-H
H - N
C
H - N'
C'
O
O'
H
H

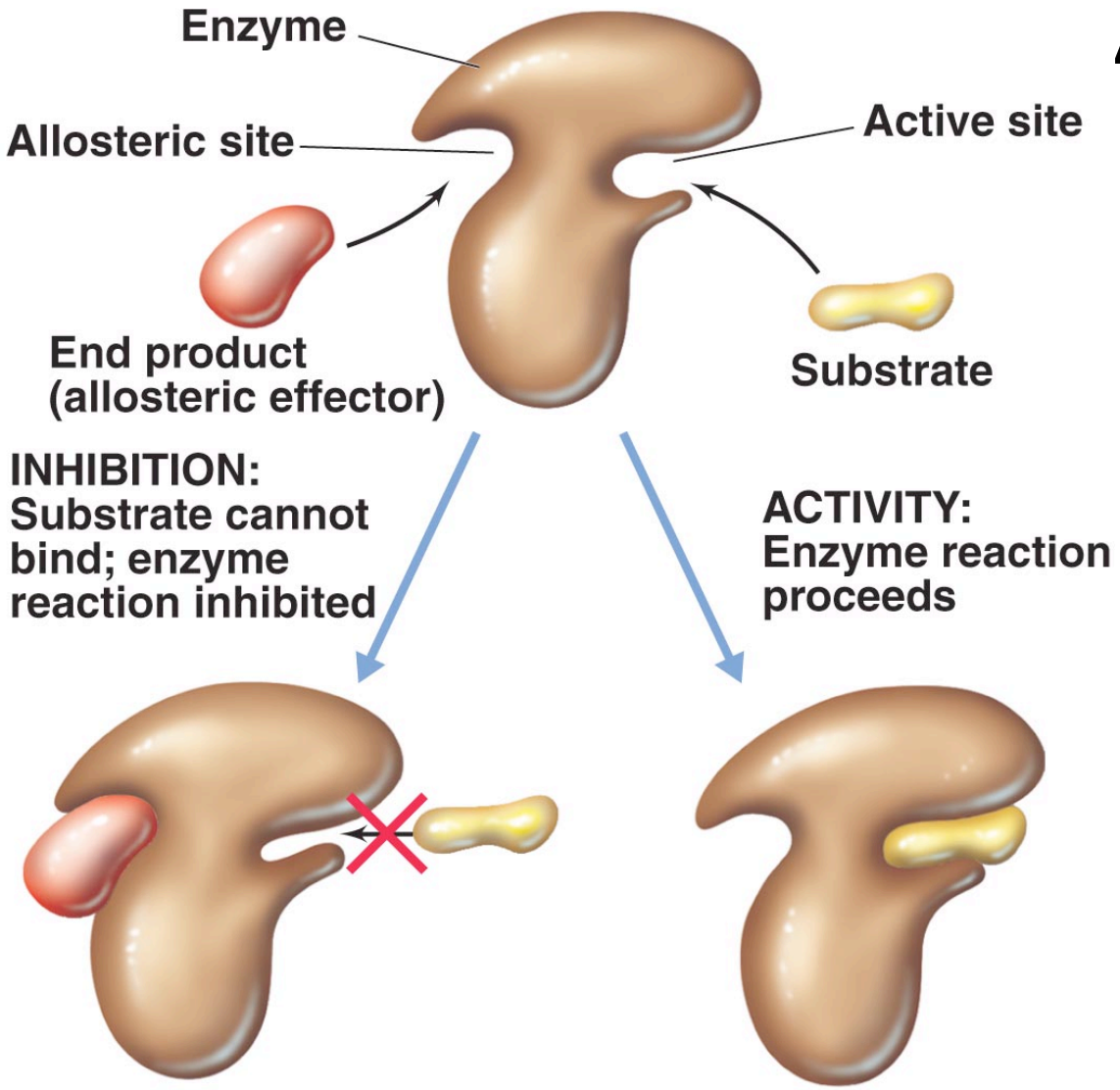


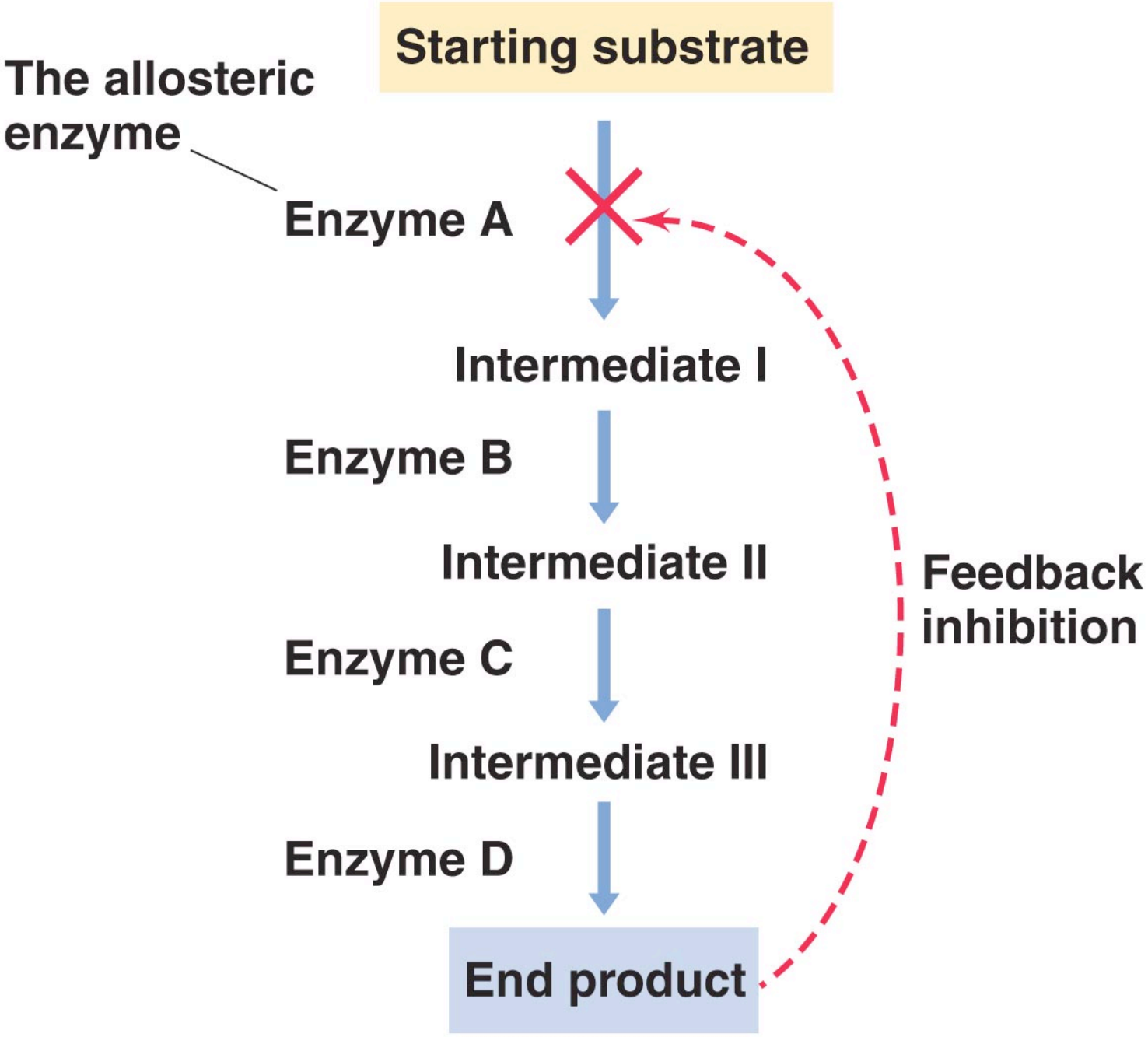

Holoenzyme
Apoenzyme
Active Site
Protein
Chains
Cofactor

Factors influencing enzyme activity

- Temperature
- pH
- Pressure
- Substrate concentration
- Post-translational regulation
  - inhibitors
    - competitive inhibition
    - allosteric inhibition
  - feedback inhibition

Allostery


Enzyme
Allosteric site
Active site
End product
(allosteric effector)
Substrate
INHIBITION:
Substrate cannot
bind; enzyme
reaction inhibited
ACTIVITY:
Enzyme reaction
proceeds


Starting substrate
The allosteric enzyme
Enzyme A
Intermediate I
Enzyme B
Intermediate II
Enzyme C
Intermediate III
Enzyme D
End product
Feedback inhibition

Catabolism

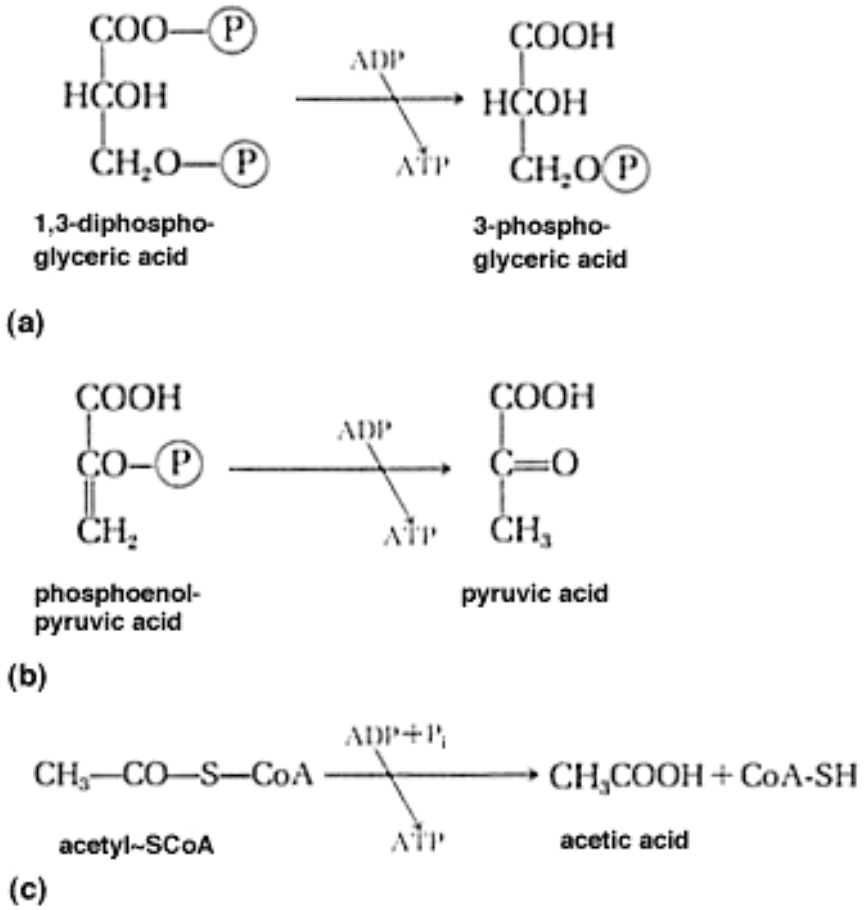
- **Goal**
  - **generate energy carriers (ATP, GTP) and electron carriers (NAD and FAD)**
- **Energy and reducing power fuel growth, repair, cell maintenance, and movement**

Energy production

- **ATP generation**
  - **substrate-level phosphorylation (SLP)**
  - **oxidative phosphorylation (ETLP)**
  - **photo-phosphorylation**

Substrate-level phosphorylation

- **SLP**
  - **synthesis of ATP directly coupled to the breakdown of high energy organic substrates**

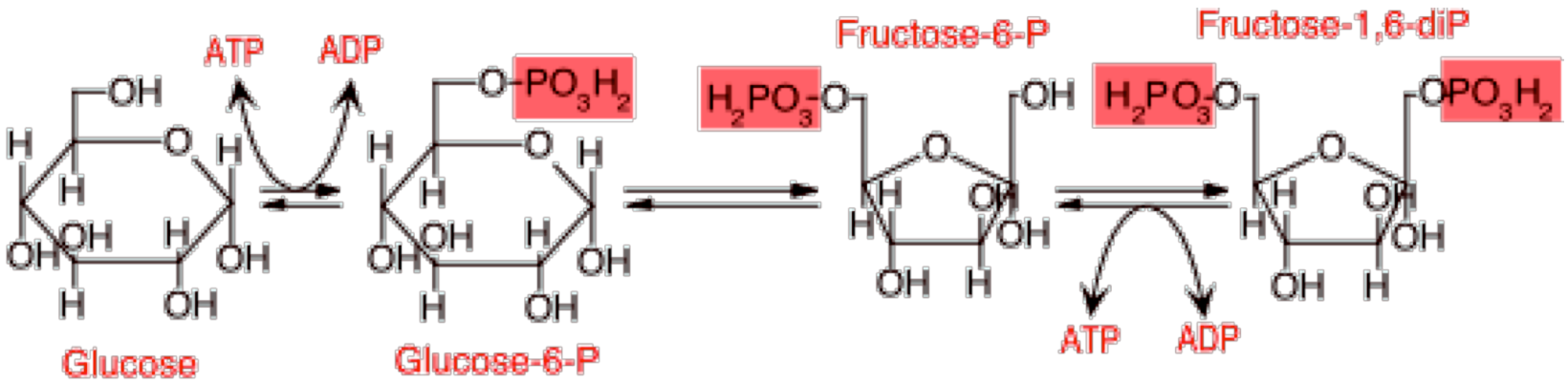


Glycolysis
(Embden-Meyerhoff-Parnas pathway)

- Most commonly used series of reactions for oxidizing glucose to pyruvate
- Glycolysis can occur in the presence or absence of oxygen
- Net gain of 2 ATP and 2 NADH (reduced electron carrier) molecules

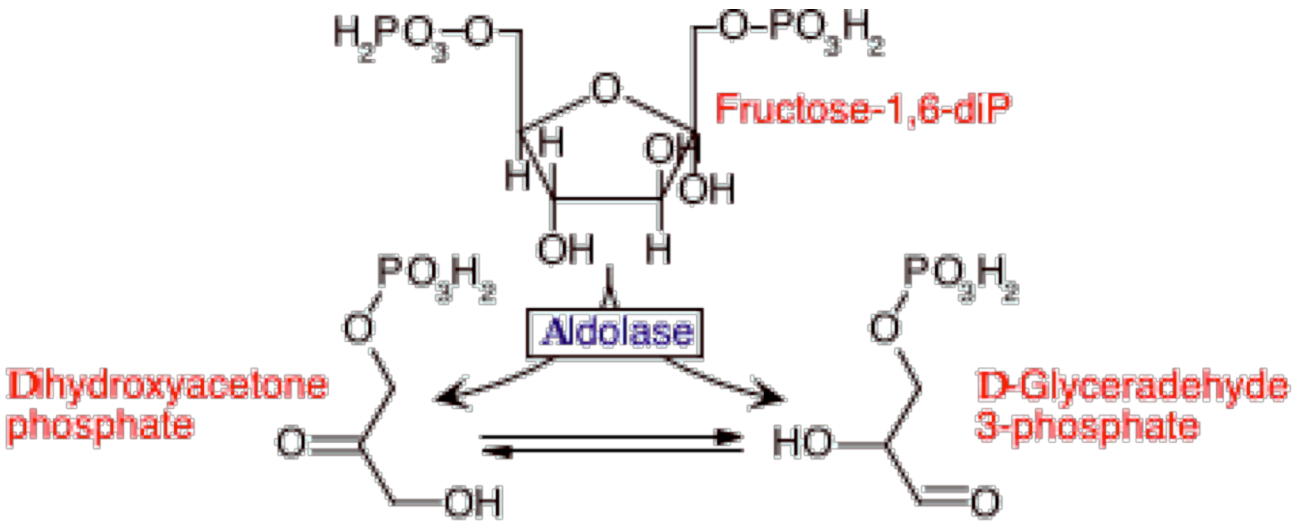
Glycolysis (cont.)

- **Activation of glucose**



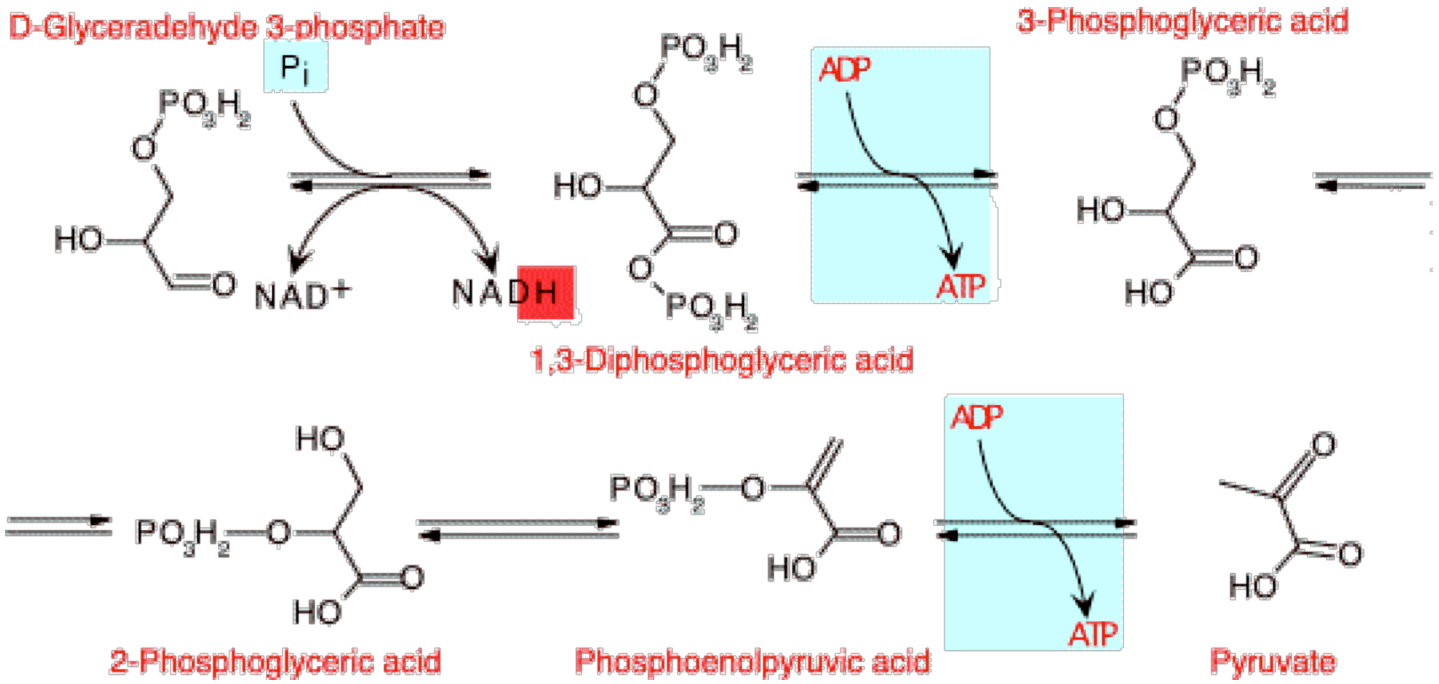
Glycolysis (cont.)

- **Hexose splitting**

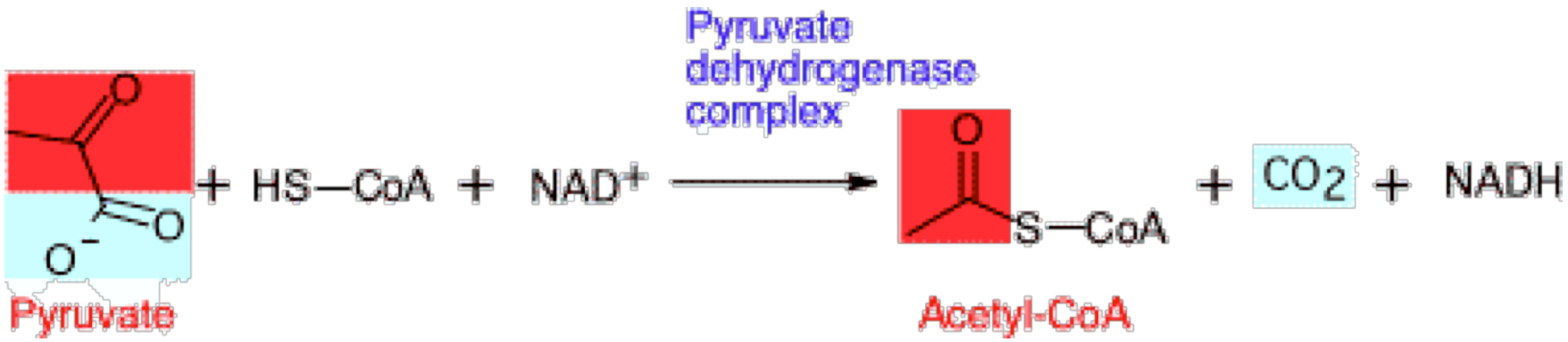


Glycolysis (cont.)

- **Energy extraction**



Coupling glycolysis to respiration



Alternatives to glycolysis

- Pentose phosphate pathway
- Entner-Dudoroff pathway

Pentose phosphate pathway

- Uses the 6 carbons of glucose to generate 5 carbon sugars and reducing equivalents (oxidative and non-oxidative branches)
- Under certain conditions it can completely oxidize glucose to CO_2 and water
- Operates exclusively in the cytosol
- Primary functions
  - generates reducing equivalents, NADPH, for reductive biosynthesis
  - provides the cell with ribose-5-phosphate (R5P) for the synthesis of the nucleotides and nucleic acids
  - metabolizes pentose sugars derived from the digestion of nucleic acids
  - rearranges the carbon skeletons of carbohydrates into glycolytic/gluconeogenic intermediates

Entner-Dudoroff pathway

- Only a few bacteria, e.g. *Zymomonas*, employ the Entner-Doudoroff pathway as a ***fermentation*** path
- Many bacteria, especially pseudomonads, use the pathway to degrade carbohydrates for ***respiratory*** metabolism
- Entner-Dudoroff pathway yields 2 pyruvic acid from glucose (same as glycolysis)
- Oxidation occurs before the cleavage, and the net energy yield per mole of glucose used is one mole of ATP

Fermentations

- Alternative to respiration
- Goal
  - NADHs need to be oxidized, “recycled”
  - pyruvate converted
- Examples
  - lactic acid fermentation
  - alcohol fermentation
  - heterofermentative microbes

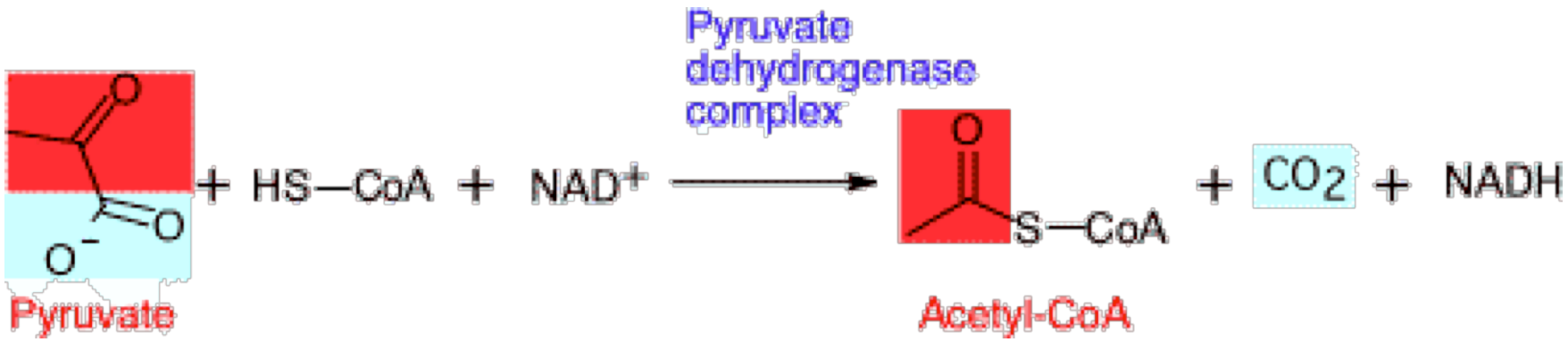
Oxidative phosphorylation

- **Electron Transfer Level Phosphorylation**
  - high energy electrons are removed from the catabolic substrate and passed on to electron carriers (often NAD or FAD)
  - carriers then transfer their electrons to an electron transport chain, which synthesizes ATP using the enzyme ATPase
  - finally, the electrons combine with O_2 (or some other terminal electron acceptor) and H^+ to form H_20 (or other reduced products)

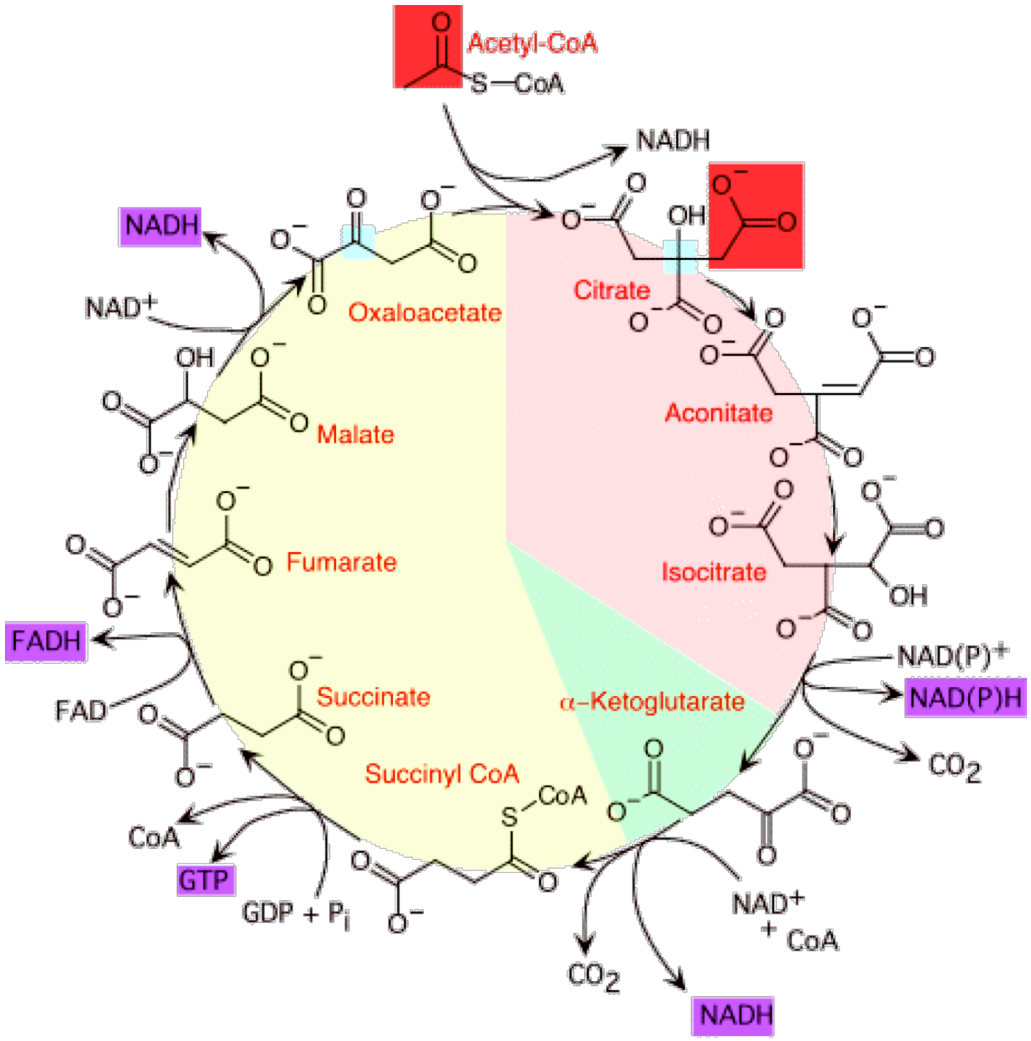
Krebs cycle

- TCA cycle is central to the metabolism of many (micro)organisms
- Many of the intermediates are also starting points (precursors) for the synthesis of cellular constituents, such as amino acids, nucleic acids and cell wall components
- During anaerobic respiration only part of the TCA cycle may operate

Coupling glycolysis to respiration

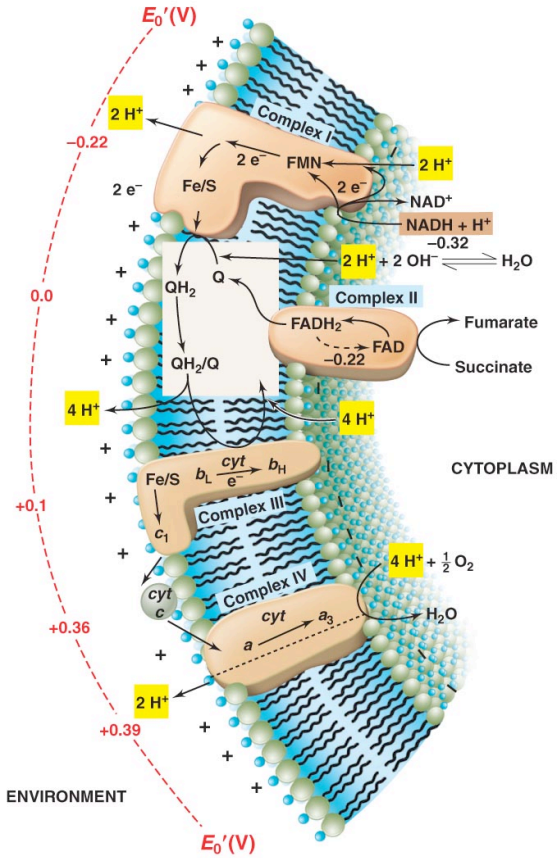


Aerobic respiration (cont.)



Electron transport system (ETS)

- **Successive electron carriers are located in close proximity so that it is easy for the electrons to pass from one complex to the next, with a minimum of delay between transfers**

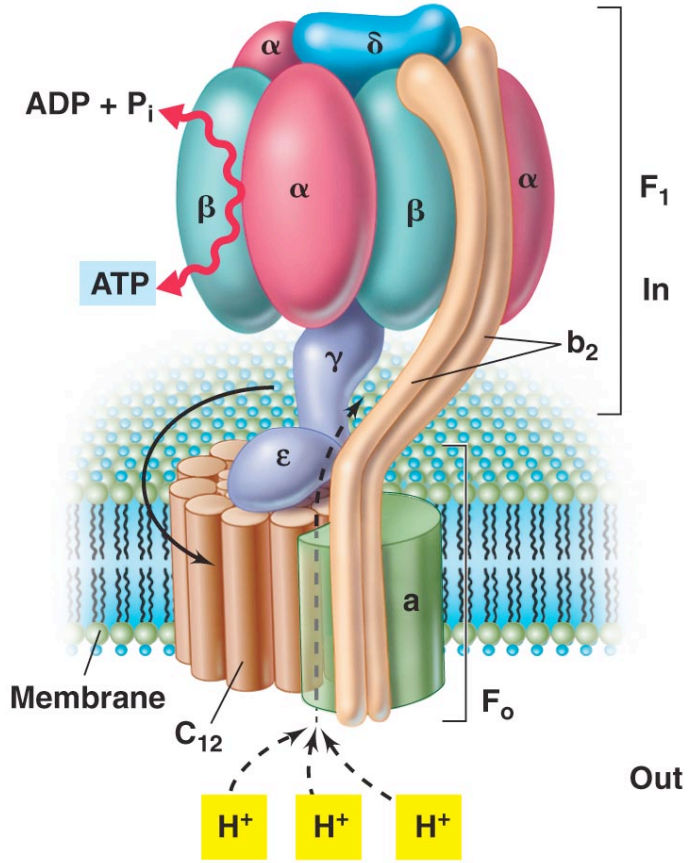


Proton motive force

- While electrons flow, protons get separated and move from one side of the membrane to the other
- Charge separation generates a transmembrane pH and proton gradient
- This build up of protons ("proton motive force") is used by the cell for many tasks, including transport, flagella movement, and ATP synthesis

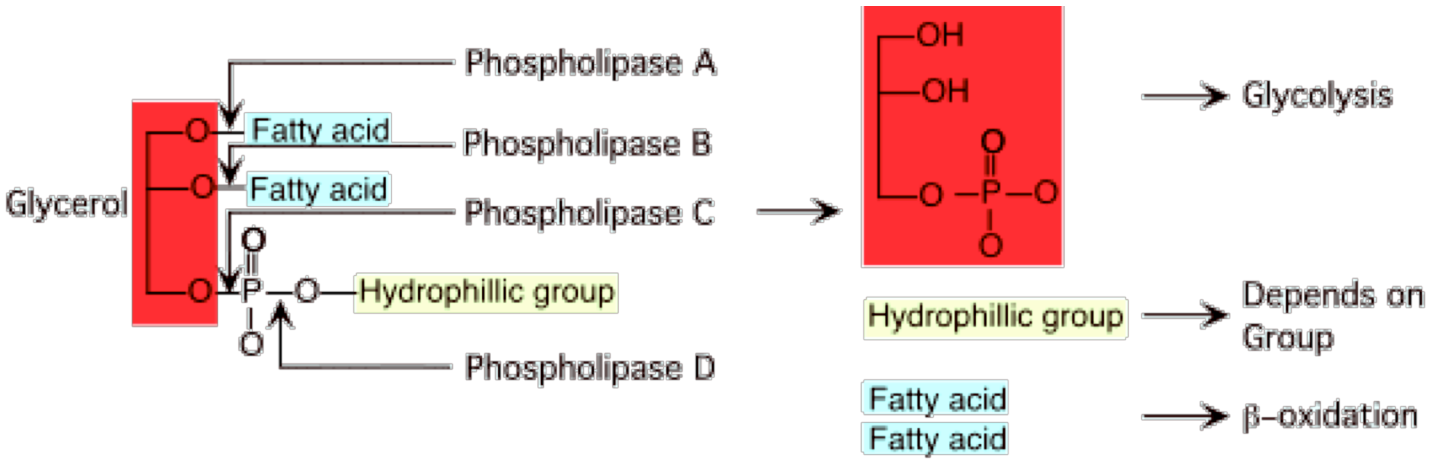
ATP synthesis

- To synthesize ATP, protons from one side of the membrane are allowed entry to the other side of the cell by “falling through” the protein complex ATPsynthase

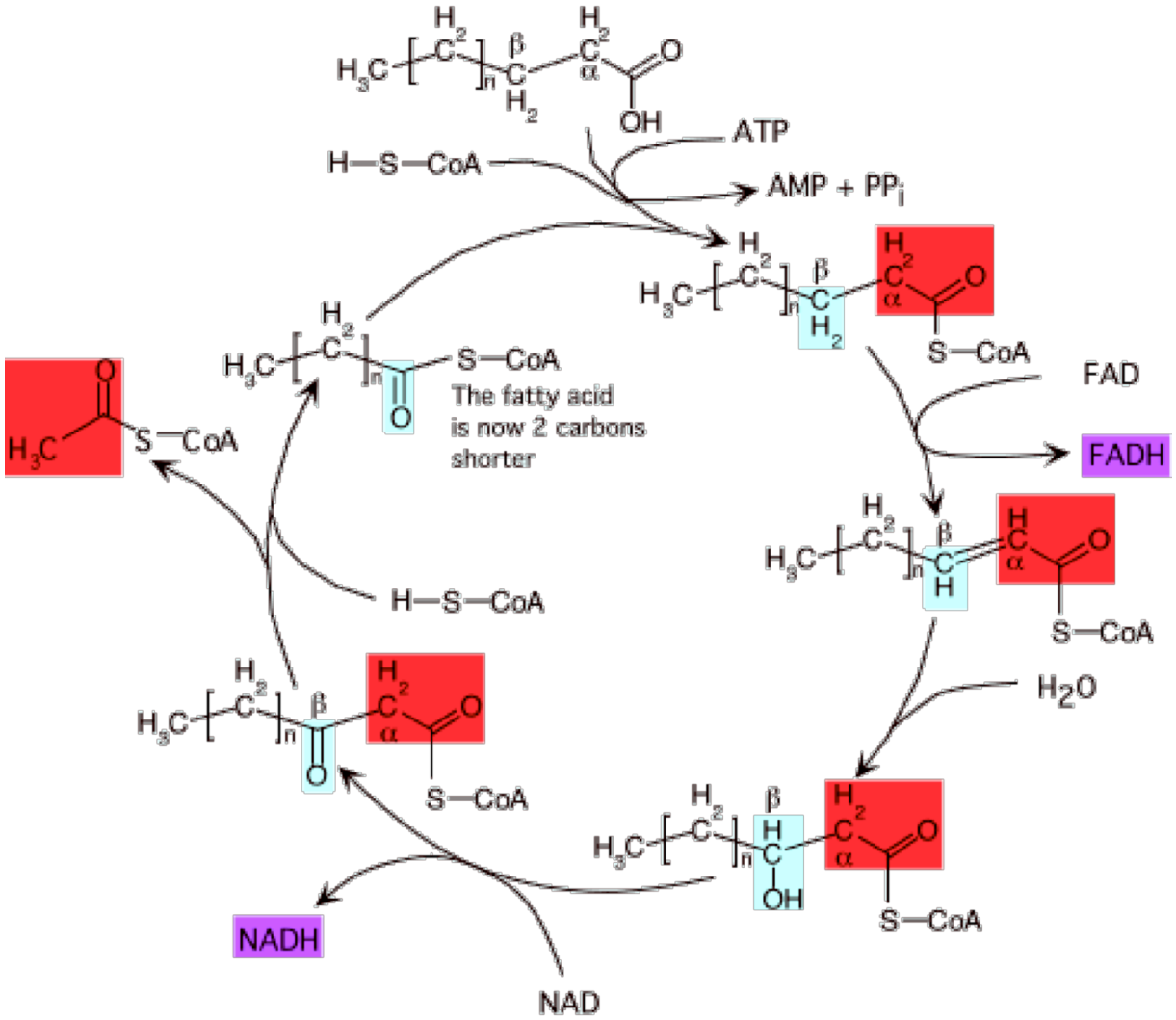


Lipid catabolism

- **Microorganisms can grow on lipids and fatty acids**
- **Extracellular lipases break down fats**

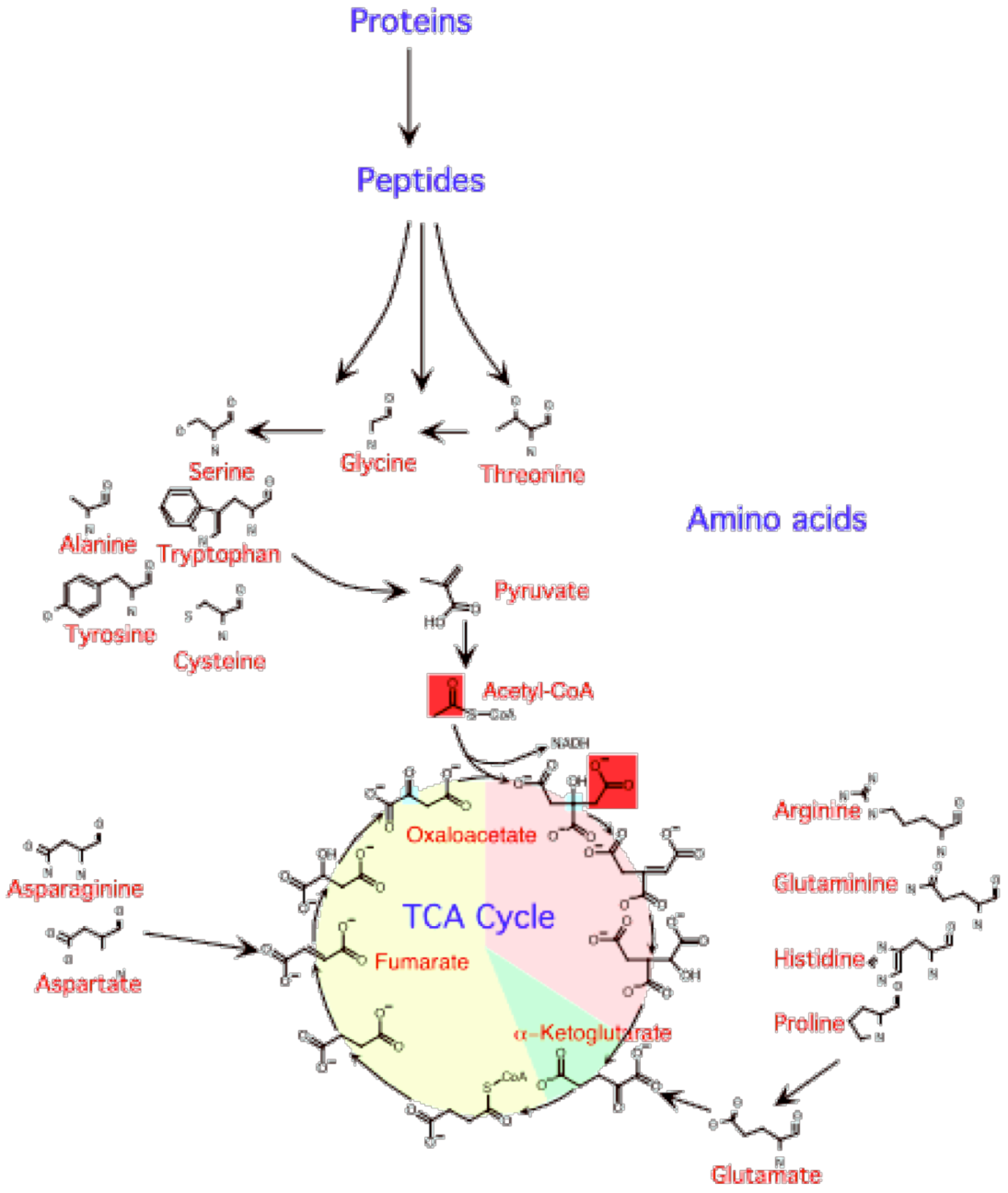


β-oxidation of fatty acids



Protein catabolism

- Extracellular proteases and peptidases break down proteins to amino acids
- AAs are converted before entering the Krebs cycle
  - deamination
  - decarboxylation
  - dehydrogenation


Proteins
Peptides
Serine
Glycine
Threonine
Alanine
Tryptophan
Tyrosine
Cysteine
Amino acids
Pyruvate
Acetyl-CoA
NADH
Oxaloacetate
TCA Cycle
Fumarate
α-Ketoglutarate
Asparaginine
Aspartate
Arginine
Glutaminine
Histidine
Proline
Glutamate

Anaerobic respiration

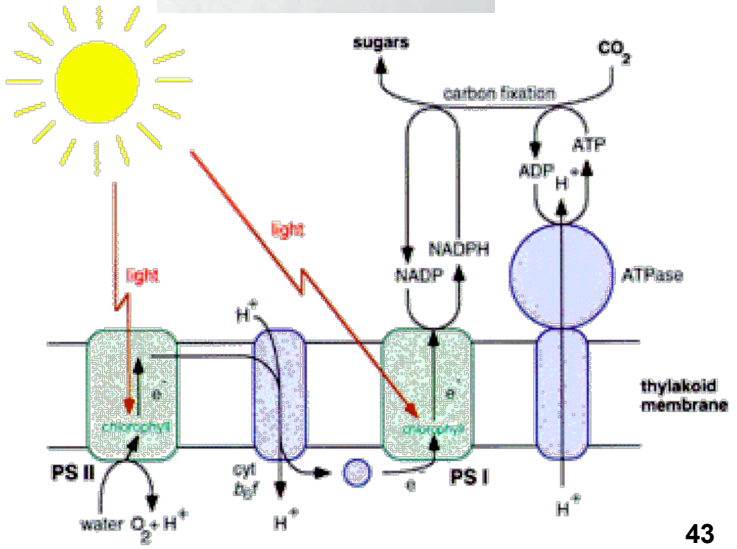
- Terminal electron acceptor is other than oxygen
- Examples
  - nitrate reduction
  - denitrification
  - iron reduction
  - sulfate reduction
  - methane production

Phototrophy

- **Conversion of light energy into chemical energy in the form of ATP**
  - **photosynthesis**
    - **chemical energy can be used in the formation of cellular material from CO_2**
      - **primary light harvesting pigment determines type**
        - » **oxygenic photosynthesis (cyanobacteria, algae, plants)**
        - » **non-oxygenic photosynthesis (purple and green bacteria)**
      - **carotenoids, phycobiliproteins**
  - **non-photosynthetic photophosphorylation**
    - **extreme halophiles developed "purple membranes"**
      - **[bacterio]rhodopsin reacts with light and forms a proton gradient allowing the synthesis of ATP**
      - **high salt environment limits oxygen availability**
      - **organisms supplement their ATP-producing capacity**

Photosynthesis

- **Light reaction**
  - catabolic component of photosynthesis
  - absorption of a quantum of light by a chlorophyll molecule causes the displacement of an electron at the reaction center
  - high potential electrons then "fall down" an ETS resulting in ATP and NAD(P)H
- **Dark reaction**
  - anabolic component that involves the fixation of CO_2
  - uses the generated ATP and NADPH to form cell carbon



Summary of catabolism

- **Fermentation**
  - electrons extracted from a relatively reduced organic compound eventually end up on a more oxidized organic molecule
  - energy yields are typically low
  - products are not fully oxidized - there is a large amount of energy left in the final product
- **Aerobic respiration**
  - electrons extracted from organic compounds
  - oxygen is the terminal electron acceptor
  - organic substrate is often completely oxidized to H_2O and CO_2
  - large amount of energy is extracted, much more than in fermentation

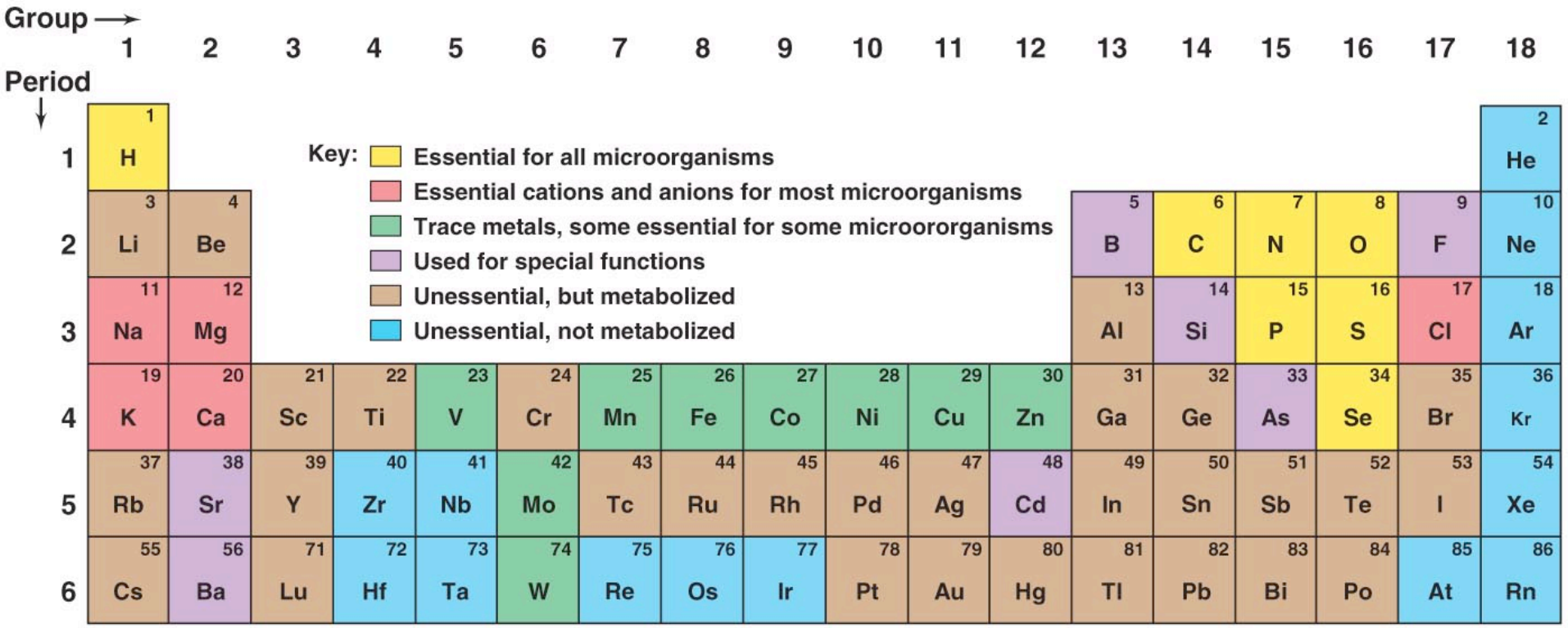
Summary of catabolism (cont.)

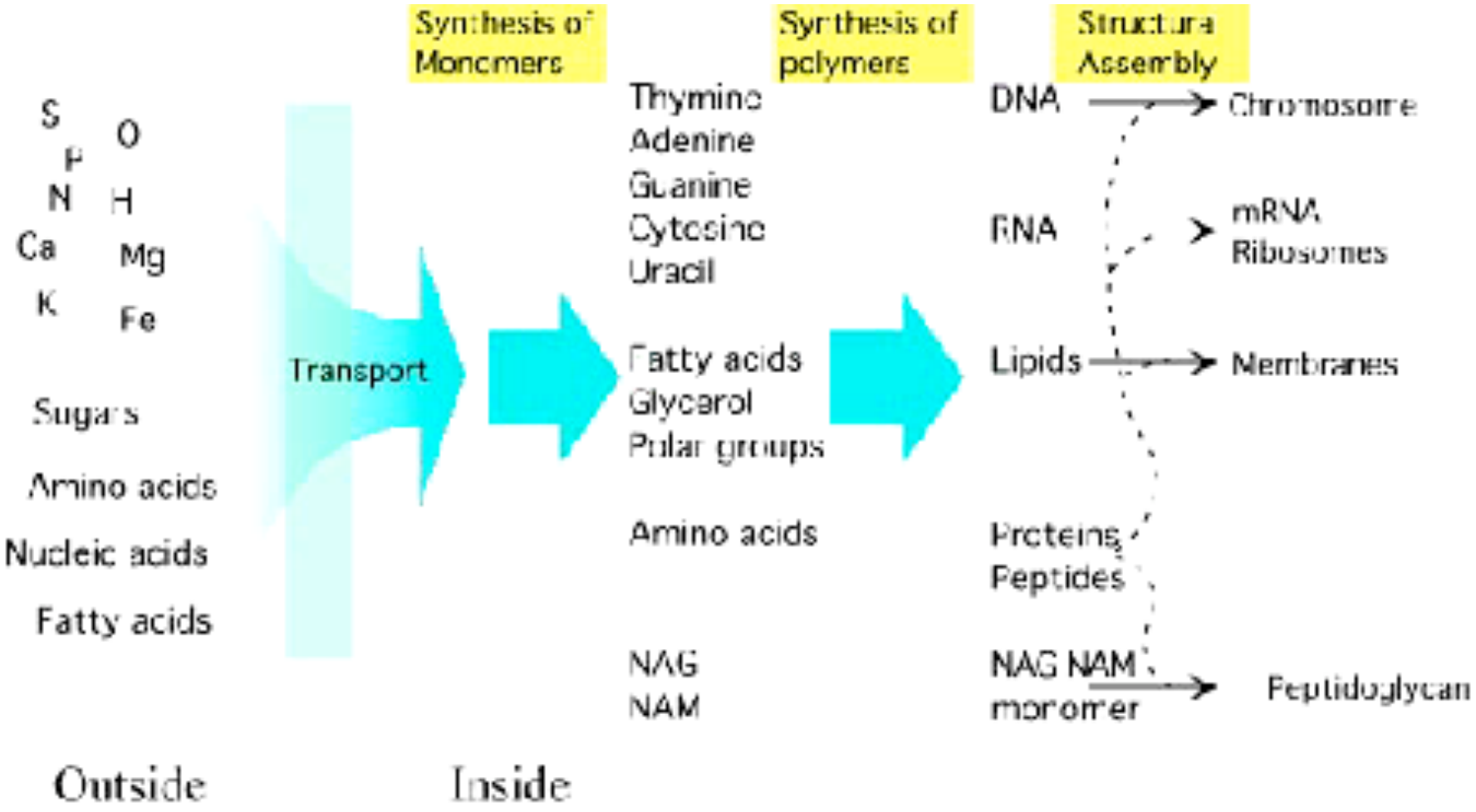
- **Anaerobic respiration**
  - electrons extracted from organic (and sometimes inorganic - lithotrophy) sources are donated to an inorganic molecule ***that is not oxygen***
  - several types of anaerobic respiration exist
  - most common terminal electron acceptors are nitrate, sulfate, and carbonate
  - anaerobic respiration typically extracts more energy than fermentation, but less than aerobic respiration
- **Phototrophy**
  - conversion of light energy into chemical energy in the form of ATP
    - photosynthesis
    - non-photosynthetic photophosphorylation

Anabolism

- General term for the synthesis of cell structures
- Phases
  - collection of elements
  - monomer synthesis
  - polymer assembly
  - organization of functional structures

Biological importance of elements




Synthesis of Moncmers
Synthesis of polymers
Structura Assembly
S
P
O
N
H
Ca
Mg
K
Fe
Sugars
Amino acids
Nucleic acids
Fatty acids
Transport
Thymine
Adenine
Guanine
Cytosine
Uracil
Fatty acids
Glycerol
Polar groups
Amino acids
NAG
NAM
DNA
Chromosome
RNA
mRNA
Ribosomes
Lipids
Membranes
Proteins
Peptides
NAG NAM
monomer
Peptidoglycan
Outside
Inside

Summary of anabolism

- Reason for doing catabolism is to drive anabolism
- Cells generate energy so that they can build more of themselves
- Anabolism costs energy
  - biological energy is in the form of ATP to drive reactions
  - NAD(P)H + H^+ to supply reducing power
- Macromolecules of the cell are synthesized from only a few simple building blocks
  - amino acids
  - sugars
  - fatty acids
  - nucleotides
  - a few other catabolic intermediates from glycolysis and the TCA cycle

Integration of metabolism

- **Catabolism and anabolism are joined trough common intermediates**
- **Amphibolic pathways**
  - **many reactions within these pathways are reversible and a cell can "decide" which way it wants to go depending on its needs at any given time**